

Glucosinolates in Turnip Tops and Roots: Cultivars Grown for Greens and/or Roots

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Abstract. Fourteen cultivars of turnip [*Brassica rapa*, *rapifera* group, also *B. campestris* L. ssp. *rapifera* (Metzg.) Sinsk.] recommended for human consumption of either tops or roots and five cultivars recommended for consumption of roots were selected to compare glucosinolate (GS) levels in tops and roots. Also, two cultivars used for animal feed were included. The study revealed significantly lower levels of 1-methylpropyl-GS and 2-hydroxy-3-butenyl-GS in tops and roots of cultivars grown for greens, compared to those used for animal feed. Contents of 1-methylpropyl-, 3-butenyl-, and 4-pentenyl-GSs were higher in turnip tops than in roots, while 2-hydroxy-3-butenyl-, 4-(methylthio)butyl-, 4-(methylsulfinyl)butyl-, 2-hydroxy-4-pentenyl-, 5-(methylthio)pentyl-, 2-phenylethyl-, 3-indolylmethyl-GSs and total GS were all higher in the roots. GS patterns for seeds tended to correlate with those of the tops.

Early workers noted that turnips were goitrogenic in rabbits (7). Glucosinolates (GSs), whose breakdown products (Figs. 1 and 2) may be goitrogenic, are present in turnips (1). Some of these products have been tested in animals. The product 5-vinyl-oxazolidine-2-thione (Fig. 2) increases thyroid and liver weights in rats (11), and thiocyanate (SCN) ion inhibits uptake of iodine by the rat thyroid (16). On the positive side, the GS hydrolysis products benzyl isothiocyanate (Table 1) and 2-phenylethyl isothiocyanate inhibit chemical carcinogenesis in rats (20).

The 1983, U.S. production of 5400 t of frozen turnip greens and 10,600 t of frozen turnip greens plus roots (15) points to the consumption of the entire plant and to the need for definitive GS analysis of the greens and roots of common cultivars.

In prior work on turnips (1), data suggested a tendency for an absence of oxazolidine-2-thiones (OZTs) in cultivars recommended for human consumption, and, for two cultivars, the GS levels of the green tops differed significantly from the levels in the roots. The present study was undertaken to study further the GS levels in turnips by analyzing tops and roots of 20 cultivars to ascertain differences in the respective plant parts. In addition, seeds of six cultivars were examined to ascertain correlations between seed GSs and those of vegetative parts.

Materials and Methods

Sample source and preparation. Seeds were sown in July 1981 or May 1982 on a farm of the Wisconsin Agricultural

Experiment Station at Madison, and plants were harvested in Sept. 1981 or July 1982. Plants were chilled during transport and then were stored at 1°C. Boiling aqueous methanol extraction (18) of 100-g wedges (diced) from three individual peeled roots and of 100-g samples of the corresponding three tops were made within 14 days of harvesting. In Apr. 1984, seeds were sown in Peoria, Ill. Plants were harvested in June, and a composite sample of tops or roots of three plants was made. Seeds were ground, defatted, and extracted with boiling water (12). Extracts were concentrated to aqueous solutions and frozen until analysis.

Methods of analysis. The aqueous extract on ion exchange resin (17) was hydrolyzed with thioglucoside glucosylhydrolase EC 3.2.3.1. (1) to produce isothiocyanates and OZTs, which were extracted into methylene chloride and analyzed by gas-liquid chromatography (2). Total glucose released enzymatically (17) from GSs was determined in seeds by a glucose-specific reagent, Glucostat X4 (Worthington) glucose reagent (19), and was calculated as total GS (18). Subsequent to our changing to Glucose Auto/Stat (Pierce), this determination in tops and roots was carried out with the Pierce glucose reagent. A calibration curve for the Pierce reagent was determined daily by using 10, 20, and 30 $\mu\text{g}\cdot\text{ml}^{-1}$ glucose standards. Thiocyanate ion produced by 3-indolylmethyl- and 3-(*N*-methoxy)indolylmethyl-GS was quantitated by an adaptation (1) of Josefsson's procedure (9). The compounds 4-methoxy-3-indolylmethyl-, 5-hydroxy-3-indolylmethyl-, and 5-methoxy-3-indolylmethyl-GS occur in brassicas (5, 13) and, if present in turnips, would be included in the SCN ion measurement. A brief outline of the analytical methodology may be found in Daxenbichler et al. (3).

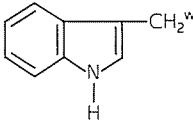
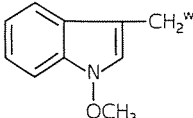
Results and Discussion

Cultivars included in this study are listed in Table 2. They have been grouped according to the analysis of their above-ground parts. Correlations between all possible pairs of cultivars were calculated from the amounts of the 11 GSs indicated in

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¹Retired.

Table 1. Glucosinolate (GS) structures.

Chemical name	Structure of R group ^z
1-Methylpropyl (413) ^y	CH ₃ CH ₂ CHCH ₃
Allyl-GS (397)	CH ₂ =CH-CH ₂
3-Butenyl-GS (411)	CH ₂ =CH-(CH ₂) ₂
4-Methylthiobutyl-GS (459)	CH ₃ -S-(CH ₂) ₄
4-Methylsulfinylbutyl-GS (475)	CH ₃ SO(CH ₂) ₄
2-Hydroxy-3-butenyl-GS (427)	CH ₂ =CH-CHOHCH ₂ ^x
4-Pentenyl-GS (425)	CH ₂ =CH-(CH ₂) ₃
5-Methylthiopentyl-GS (473)	CH ₃ -S-(CH ₂) ₅
5-Methylsulfinylpentyl-GS (489)	CH ₃ -SO-(CH ₂) ₅
2-Hydroxy-4-pentenyl-GS (441)	CH ₂ =CH-CH ₂ -CHOH-CH ₂ ^x
2-Phenylethyl-GS (487)	C ₆ H ₅ CH ₂ CH ₂
3-Indolylmethyl-GS (487)	
3-(<i>N</i> -Methoxy)indolylmethyl-GS (516)	

^zSee Fig. 1^yValues in parentheses indicate the molecular weight of the GS as the potassium salt.^xCyclic oxazolidine-2-thione forms if GS is hydrolyzed under conditions expected to yield isothiocyanate.^wSCN ion forms as a decomposition product from unstable isothiocyanate.

Table 3. In effect, the relative amounts of the GSs (the GS pattern) were compared for all cultivars, as was done previously for cabbage (12). Groups then were formed that contained correlation coefficients of 0.74 or higher among themselves, but lower correlations with other cultivars. The value 0.74 corresponds to the 1% significance level in the simple linear correlation (*r*) based on 11 points. Likewise, the seeds of six of these cultivars fell into two groups (A and B), as indicated. There is a decreasing progression of correlation coefficients between the groups when moving from group I to group IV, which results in group IV being the least correlated with group I. Such groupings elucidate chemical interrelationships of cultivars and may be useful to plant breeders. Published information in commercial seed catalogs and private communications with seed companies provided the recommended use information for the given cultivars shown in Table 2.

The tops of several cultivars used either for greens or for both greens and roots had different GS patterns than the tops of cultivars produced for roots only. One very noticeable characteristic of group I (see Table 3) is the low levels of 2-hydroxy-3-butenyl- and 2-hydroxy-4-pentenyl-GS, both of which produce OZTs upon hydrolysis. When the data reported here were combined with our previous work (1, 8), it was found that, over 2 to 3 years of cropping, 'Tokyo Market', 'Tokyo Market Express', 'Tokyo Market Second Early', and 'Tokyo Top' produced no detectable amounts of either of these components in tops or roots. 'Tokyo Cross' had none in the tops and only up to 5 μmol/100 g in the roots. 'Crawford' and 'Showtop', grown for 1 year, likewise had none in the tops (and 'Showtop' only 4 μmol/100 g in the roots), bringing the number of cultivars to

seven that we have found to have zero levels in the tops. For these same two to three crop years, the tops of 'Presto', 'Just Right' and, 'Shogoin' had zero to low levels (up to 6 μmol/100 g) of these two components, while the corresponding peeled roots of 'Presto' had none and those of 'Just Right' and 'Shogoin' fell into the 7–78 μmol/100 g range. [The GS contents of root peelings of 'Presto', 'Just Right', and 'Shogoin' were very similar to the corresponding values in the roots (D.G. Carlson, unpublished data).] On the other hand, 'Roots' and 'White Egg', both recommended only for roots, had 19 and 26 μmol/100 g, respectively, in the tops (the highest levels in group I cultivars) and 36 and 74 in the roots. 'White Knight' tops were most highly correlated (*r* = 1.00) with 'Just Right' and 'Shogoin'.

Fenwick and Griffiths (4) have used sensory analysis to show that 5-vinyl-OZT, the hydrolysis product of 2-hydroxy-3-butenyl-GS (Fig. 2), is a bitter principal of cooked brussels sprouts. Thus, there is a chemical basis underlying the use of terms such as "fine tasting," "mild," and "without bitterness" used in seed catalogs to describe group I cultivars.

Compared to group I, the relative amounts of 4-butenyl-GS and 4-pentenyl-GS in group II are reversed, and the levels of 2-hydroxy-3-butenyl-GS, 2-hydroxy-4-pentenyl-GS, and the total GSs are increased in this group containing two cultivars recommended for tops and one used primarily as stock feed. In group II, the 4-pentenyl- and 5-(methylthio)pentyl-GS content of 'Charlestowne' roots (165 and 118 μmol/100 g, respectively), and 4-pentenyl-GS in the tops (179 μmol/100 g) were 1.6 times greater than the highest corresponding values of group I members. In tops of group III, 'Royal Crown' values are at the low end of the range. 'Purple Top White Globe' (also in group III) and 'Royal Crown', grown primarily for roots, have higher levels of 1-methylpropyl-, 2-hydroxy-3-butenyl-, and 2-hydroxy-4-pentenyl-GS, and lower levels of 4-butenyl-GS compared to the majority of the 14 group I cultivars.

Although there is reported evidence of fairly consistent GS profiles across years and sites (6) for a given cultivar, there is some shifting of these cultivars in their correlation among themselves for different years or locations. Therefore, our grouping, where possible, has been based on the 1982 crop, since more cultivars were grown and compared in that year than in 1981.

When the cultivars with edible roots were groups (Tables 2 and 3) on the basis of the GS pattern in their roots, all those recommended for both roots and greens were in the same group, with the exception of 'Charlestowne' and 'Shogoin'. Thus, both root and top content are distinguishable for cultivars used for the whole plant vs. those used only for roots. Additionally, a statistical analysis of turnips grown in 1981 and 1982 showed that, for tops and for roots in each year, 1-methylpropyl- and 2-hydroxy-3-butenyl-GS were both significantly lower in the cultivars grown for tops or for both tops and roots than in those grown only for roots.

The levels of total GS in the turnip seeds are 30–110 times those in the vegetative parts; hence, the compounds in seeds are reported as millimoles rather than micromoles. In progressing from group I to group IV, the trend of compound concentrations in vegetative parts is toward reduced relative amounts of 3-butenyl-GS and concomitant increasing amounts of 2-hydroxy-3-butenyl-GS. The corresponding seeds show this same trend, which results in two groups of seeds. Thus, 'Just Right' seed (group A) has 87% of its total GS as 3-butenyl-GS and 0.4% 2-hydroxy-3-butenyl- plus 2-hydroxy-4-pentenyl-GS. In contrast, 'Purple Top Strap Leaf' (seed group B) has 31% and 46%,

Table 2. Cultivars listed in correlation coefficient groups^z for tops.

Cultivars	Seed group ^z	No. plants analyzed						Recommended for				
		1981		1982		1984		Greens	Greens and roots	Roots	Animal feed	
		T ^y	R	T	R	T	R					
Group I ^z												
1) Alltop ^x				2	2			*				
2) Crawford				3	3			*				
3) Just Right ^x	A	3	3						*			
4) Presto		3	2	3	3				*			
5) Roots				6	6					*		
6) Shogoin		3	3						*			
7) Showtop ^x				3	3				*			
8) Tokyo Cross ^x				6	6				*			
9) Tokyo Market	A	1	3	3	3				*			
10) Tokyo Market Express ^x		3	3						*			
11) Tokyo Market Second Early		3	3	3	3				*			
12) Tokyo Top ^x		3	3	3	3				*			
13) White Egg		3	2	3	3					*		
14) White Knight ^x						3	3		*			
Group II												
15) Charlestowne				6	6				*			
16) Cowhorn	A			3	3						*	
17) Seven Top						3		*				
Group III												
18) Purple Top White Globe	A	3	3	3	3					*		
19) Royal Crown ^x				6	6					*		
Group IV												
20) Purple Top Strap Leaf	B	3	3	3	3	3	3			*		
21) Tigra	B										*	

^zAny 2 cultivars within a group have a correlation coefficient of 0.74 or greater.^yT = tops; R = roots.^xHybrid. Remaining cultivars are open-pollinated.

respectively, of these GSs. The corresponding values for the tops are 69% and 0% for 'Just Right', and 2% and 27% for 'Purple Top Strap Leaf'.

GS patterns of the seed of six cultivars (Table 3) were compared with those of the corresponding tops and roots for these cultivars. [Previously published data on 'Tigra' tops and roots (1) also were included.] As observed for cabbage seeds and heads (12), there was a tendency for similar GS patterns of turnip seeds and tops. There was high correlation between the tops and seeds of 'Just Right', 'Tokyo Market', and 'Cowhorn', but little correlation for 'Purple Top White Globe', 'Purple Top Strap Leaf', and 'Tigra'. There was a lower correlation in turnip seeds vs. roots, partly because the 2-phenylethyl-GS in seeds and tops lay in the range of 1–10% of the total GSs, whereas in the roots it ranged from 17–45%.

In order to determine whether the levels of GS components in the tops differed from those in the roots, an analysis of variance was carried out on data from each of the two years (Table 4). When the GS levels in the tops were tested against those in the roots for all cultivars over both years, a significant difference (with 92 df) was seen for the level of every GS in the tops vs. that in the roots. The difference was highly significant for all but 5-(methylsulfinyl)pentyl-GS. The simple alkyl GS (1-methylpropyl-GS) and simple alkenyl GSs (3-butenyl- and 4-pentenyl-GSs), were concentrated in the tops, while other GSs and the total GS were at higher levels in the roots. These trends correspond with our previous observations and conclusions (1) with the exception of the 3-indolylmethyl-GSs, which had shown

a higher level in the tops in the limited sampling taken.

Relationships between GSs were revealed when correlations were computed between all possible pairs of GSs. A high correlation (0.83, $P < 0.01$) was observed between 5-(methylthio)pentyl- and 5-(methylsulfinyl)pentyl-GS for roots of 5 cultivars recommended primarily for roots (plants grown 1–4 years). A significant ($P < 0.05$) negative correlation was seen between 3-butenyl- and 2-hydroxy-3-butenyl-GS in seeds of six cultivars. This negative correlation is not surprising, considering that these two compounds may have the same precursor (10). Likewise, in roots of 12 cultivars recommended for tops or for tops and roots (1–4 crop years) there was high correlation (≥ 0.79 , $P < 0.01$) between 4-pentenyl- and 5-(methylthio)pentyl-GS, and also between 2-phenylethyl-GS and each of these 2 GSs. These types of correlations may prove useful in the growing body of data on GS biosynthesis (14).

Information on the variability of GSs in turnips may be helpful in understanding the chemical basis of genetic diversity in this crop, and may provide information for lowering the concentrations of 2-hydroxy-3-butenyl- and 3-indolylmethyl-GSs in future cultivars through plant breeding.

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Table 3. Glucosinolate (GS) content of tops and seed in cultivar groups.

Glucosinolate	Tops, $\mu\text{mol}/100 \text{ g}$ fresh wt ^z				Seeds, mmol/100 g defatted meal ^z	
	Group ^y				A (4)	B (2)
	I (14) ^x	II (3)	III (2)	IV (1)		
1-Methylpropyl (tops) or allyl (seeds)	5 (0–27) 103	13 (1–37) 67	28 (26–29) 44	10 3	0.06 (0.01–0.2) 8.9	0.4 (0–0.8) 3.0
3-Butenyl	(38–181) 3	(14–96) 13	(13–76) 17	16	(4.0–10.8) 0.4	(2.6–3.4) 3.6
2-Hydroxy-3-butenyl	(0–25) 0.8	(6–19) 0.3	(12–22) 0	0.8	(0.06–0.9) 0.03	(3.1–4.2) 0.1
4-(Methylthio)- and 4-(methylsulfinyl)butyl	(0–10) 58	(0–0.5) 138	 137	 16	(0–0.06) 1.6	(0–0.2) 1.6
4-Pentenyl	(20–112) 0.7	(19–217) 5	(68–207) 13	13	(0.6–2.8) 0.08	(1.0–2.2) 0.7
2-Hydroxy-4-pentenyl	(0–6) 1	(2–10) 2	(11–15) 0.8	1	(0–0.2) 0.03	(0.6–0.7) 0.03
5-(Methylthio)pentyl	(0–15) 2	(0.8–3) 13	(0.5–1) 2	17	(0–0.1) 0	(0–0.06) 0
5-(Methylsulfinyl)pentyl	(0–8) 7	(4–23) 16	(1–3) 31	4	0.3	0.2
2-Phenylethyl	(2–23) 8	(5–21) 34	(25–37) 6	30	(0.2–0.5) 0.6	(0.2) 0.7
3-Indolylmethyl ^w	(0–25) 186	(12–68) 296	(4–7) 292	114	(0.5–0.8) 13.1	(0.7–0.8) 13.1
Total GS ^v	(80–292)	(97–474)	(194–389)		(10.0–15.6)	(11.7–14.4)

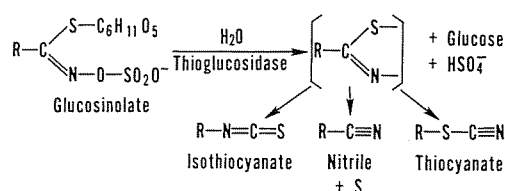
^zMeans above, ranges below and in parentheses.^yAny two cultivars within a group have correlation coefficients of 0.74 or greater.^xNumber of cultivars in the group.^wIncludes any 3-indolylmethyl-GS measured by the SCN ion procedure.^vCalculated from glucose-released enzymatically based on an average molecular weight of 457 for GSs.

Fig. 1. Major product classes from the enzymatic hydrolysis of glucosinolates. See Table 1 for R group listing.

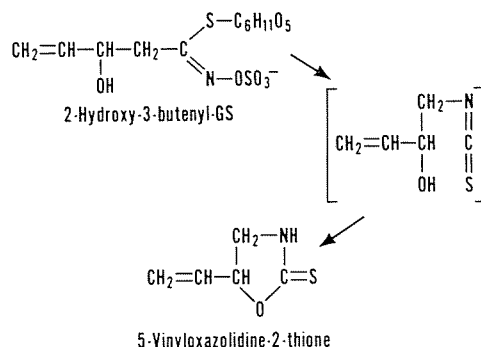


Fig. 2. Formation of an oxazolidine-2-thione from enzymatic hydrolysis of a glucosinolate common in crucifer vegetables.

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Table 4. Glucosinolate (GS) content of tops vs. roots.

	Glucosinolate ($\mu\text{mol}/100\text{g}$) fresh wt							
	1981 (7 cultivars)				1982 (12 cultivars)			
	Tops		Roots		Tops		Roots	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1-Methylpropyl	6	2	4	4	9	7	3	3
3-Butenyl	53	25	37	21	92	74	46	31
2-Hydroxy-3-butenyl	4	1	19	12	8	9	24	19
4-(Methylthio)butyl	0	0	7	3	0.01	0.05	18	17
4-Pentenyl	34	16	23	9	83	48	47	24
2-Hydroxy-4-pentenyl	2	0.8	4	4	4	5	9	7
5-(Methylthio)pentyl	0.3	0.2	12	6	0.5	0.7	34	16
2-Phenylethyl	4	3	81	28	15	8	130	49
3-Indolylmethyl ^y	8	11	20	21	9	5	22	13
Total GS ^x	148	51	198	79	221	86	291	70

^zSD between samples were pooled over all cultivars with 20 df for 1981 and 47 df for 1982.

^yIncludes any 3-indolylmethyl-GS measured by the SCN ion procedure.

^xGS value calculated from glucose released.

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